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Toxic effects of digoxin on heart and troponin level in laboratory rat

تأثيرات السمية لديجوكسين على القلب ومستوى التروبونين في الفئران المختبرية

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Abstract

The current study was conducted at the college of veterinary medicine, university of basrah in the periods extended from 22/10/2016 to 22/1/2017. Digoxin is an important cause of poisoning. It is prescribed and widely used to patients with heart failure. Digoxin toxicity can emerge during long-term therapy as well as after an overdose. Therefore, the study is designed to report the toxic effects of digoxin on heart histologically and biochemically by measuring troponin enzyme level in the serum. Maximum toxic dose (MTD) determined by using 2 rats dosed orally until clinical signs of toxicity became prominent at 30mg for each rat and considered as MTD. The chronic toxicity study was carried out on 48 adult rats divided into 4 groups, each group includes 12 rats. Control (G1) receive distilled water, Low dose (G2) dosed with (1.5mg/kg) digoxin, Intermediate dose (G3) dosed with (3mg/kg) digoxin and High dose (G4) dosed with (6mg/kg) digoxin by oral gavage for 90 days. At the end of experiments all animals were sacrificed and blood sample were collected for estimation of biochemical parameter of rat. Result reveal histopathological change of heart ranges from massive areas of degeneration of myocyte with homogenous cytoplasm to vacuolation of myocytes. Also show that a significant ($P \leq 0.05$) increase in serum troponin level in High dose (G4) group when compared with other study groups. We conclude that high dose digoxin has toxic effects on heart.

الخلاصة

أجريت الدراسة الحالية في كلية الطب البيطري بجامعة البصرة في الفترات الممتدة من 2016/10/2 إلى 2017/1/22. الديجوكسين هو سبب مهم للتسمم. وهو يوصف ويستخدم على نطاق واسع للمرضى الذين يعانون من قصور القلب. يمكن أن تظهر السمية الديجوكسين خلال العلاج على المدى الطويل وكذلك بعد جرعة زائدة. لذلك، تم تصميم الدراسة للإبلاغ عن الآثار السامة للديجوكسين على القلب نسيجيا وكيميائيا بيولوجيا عن طريق قياس مستوى الانزيم التروبونين في المصل. الحد الأقصى للجرعة السامة (MTD) التي تحددها استخدام فأرتين جرعت فمويا حتى أصبحت العلامات سريرية للسمية بارزة عند 30mg لكل فأر واعتبرت ك MTD. أجريت دراسة السمية المزمنة على 48 فئران البالغة مقسمة إلى 4 مجموعات، كل مجموعة تضم 12 فأر. مجموعة السيطرة (G1)تأخذ ماء مقطر والمجموعة الثانية (G2)جرعت بالجرعة منخفضة من (ديجوكسين 1.5 ملغم / كغم)، والمجموعة الثالثة (G3)جرعت بالجرعة المتوسطة من ديجوكسين (3 ملغم / كغم) ومجموعة الرابعة (G4) جرعت بالجرعة العالية ديجوكسين 6 ملغم / كغم عن طريق الفم بأنبوب لمدة 90 يوما. في نهاية التجارب تم التضحية بجميع الحيوانات وثم جمعت عينات الدم لتقدير قيم البيو كيميائية للفئران. النتيجة كشفت التغيير النسيجي المرضي للقلب يتراوح من المناطق الضخمة من الخلية العضلية المتحللة مع السيتوبلازم متجانس الى فجوات في الخلايا العضلية. كذلك اظهرت زيادة معنوية (($P \leq 0.05$) في مستوى مصلي للتروبونين في مجموعة الرابعة (G4) جرعة عالية عند مقارنتها بمجموعات الدراسة الاخرى. نستنتج أن جرعة عالية من الديجوكسين له آثار سامة على القلب.

Introduction

Cardiac glycosides are an important cause of poisoning; toxicity can occur during long-term treatment as well as after an overdose. Arrhythmias are the major adverse effect of digoxin which may lead to hospitalization, morbidity and even death **[1].** Digoxin is the most common cardio tonic medications still in use around the world **[2].** Therefore, our study is designed to determine the toxic effects of long term use, high dose administration of digoxin on heart. Digoxin is a purified cardiac glycoside extracted from the leaves of the foxglove plant **[3].** Digoxin increases intracellular calcium in myocardial cells indirectly, by inhibiting the sodium–potassium pump in the cell membrane. Increased intracellular calcium increases cardiac contractility **[4, 5].**

Materials and Methods

Forty-eight adult rats (24 male and 24 female rats) weighing (170 ± 40g) were used in the study and divided into 4 groups including, High dose (G4), Intermediate dose (G3), Low dose (G2) and Control (G1) group. Each group consists of 12 rats (6male and 6 female rats) and dosed with different dose of digoxin. High dose group receive (6mg/kg) digoxin body weight of rat, that is 1/5 MTD **[6]**, Intermediate dose group receive (3mg/kg) digoxin body weight of rat, that is 1/2 High dose, Low dose group receive (1.5mg/kg) digoxin body weight of rats, that is 1/2 Intermediate dose and the Control group receive distilled water. After 90 days' study, all animals were sacrificed and blood sample were collected and centrifuged at 3000rpm for 15min. and the serum collected in Eppendorf tube and stored at -20C^{O} for laboratory analysis of troponin enzyme. The concentration of troponin enzyme was measured at the end of experiment by fluorescence immunoassay **[7]** using ichroma^{TM} Tn-I kit (Boditech Med Incorporated).

Data were expressed as mean ± standard deviation and analyzed statistically using the Microsoft Program SPSS version 11. Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.05$). Specific group differences were determined using least significant differences (LSD).

Results

Histopathological change of heart ranges from massive areas of degeneration of myocyte with homogenous cytoplasm as pale areas somewhat grayish in color as in figure (1) to degeneration due to vacuolation of myocyte shows in figure (2). There is also sub capsular congestion of vessels and sub capsular fibrosis in figure (3,4). Note presence of adipose tissue and groups of ganglia like cells in pericardial region shows in figure (5,6).

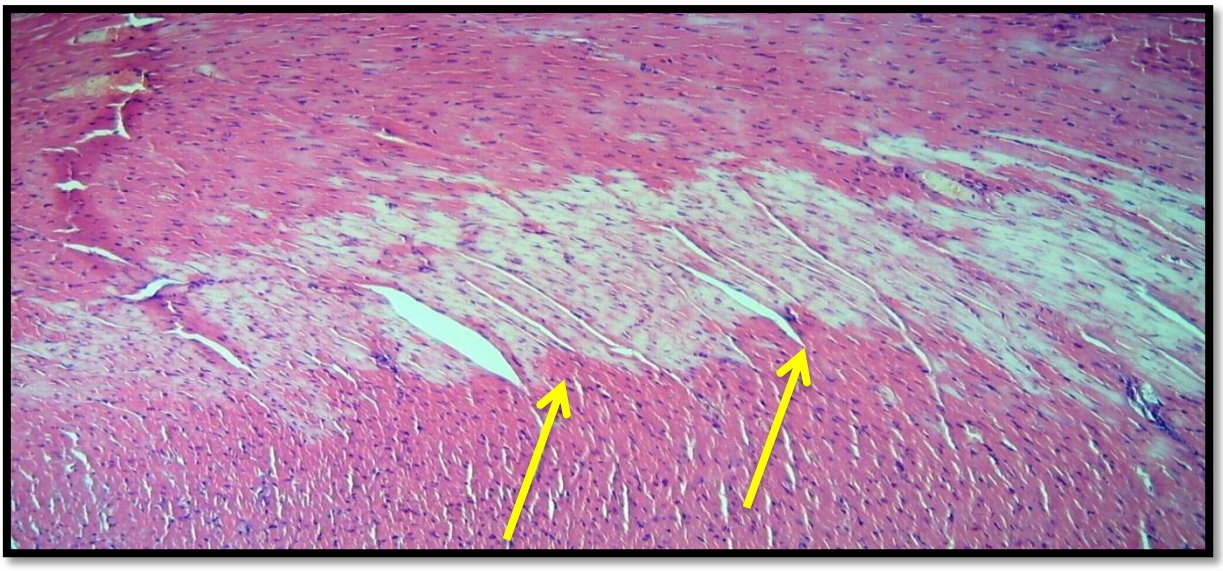

Figure (1): Heart muscle of male rat shows pale areas of degenerate myocardial muscle cells with homogenous cytoplasm somewhat grayish in color. H&E stain 100X.

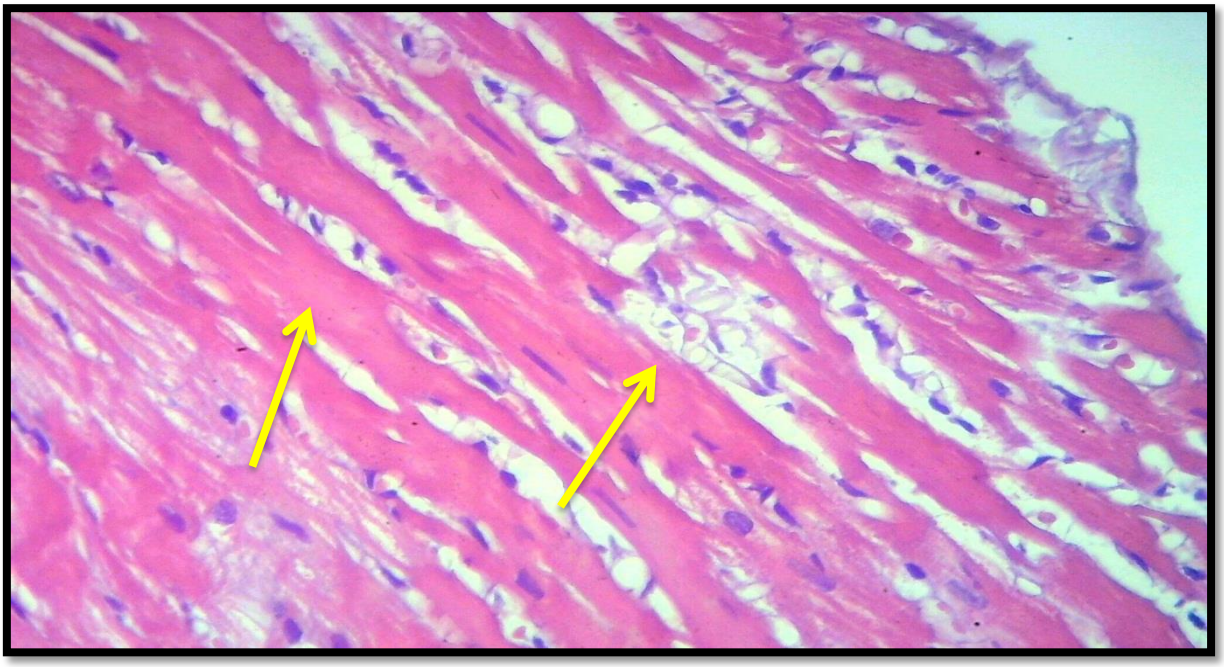

Figure (2): Heart muscle of male rat shows areas of degenerate vacuolated myocardial muscle cells, early degeneration of cardiac muscle cells. H&E stain 400X.

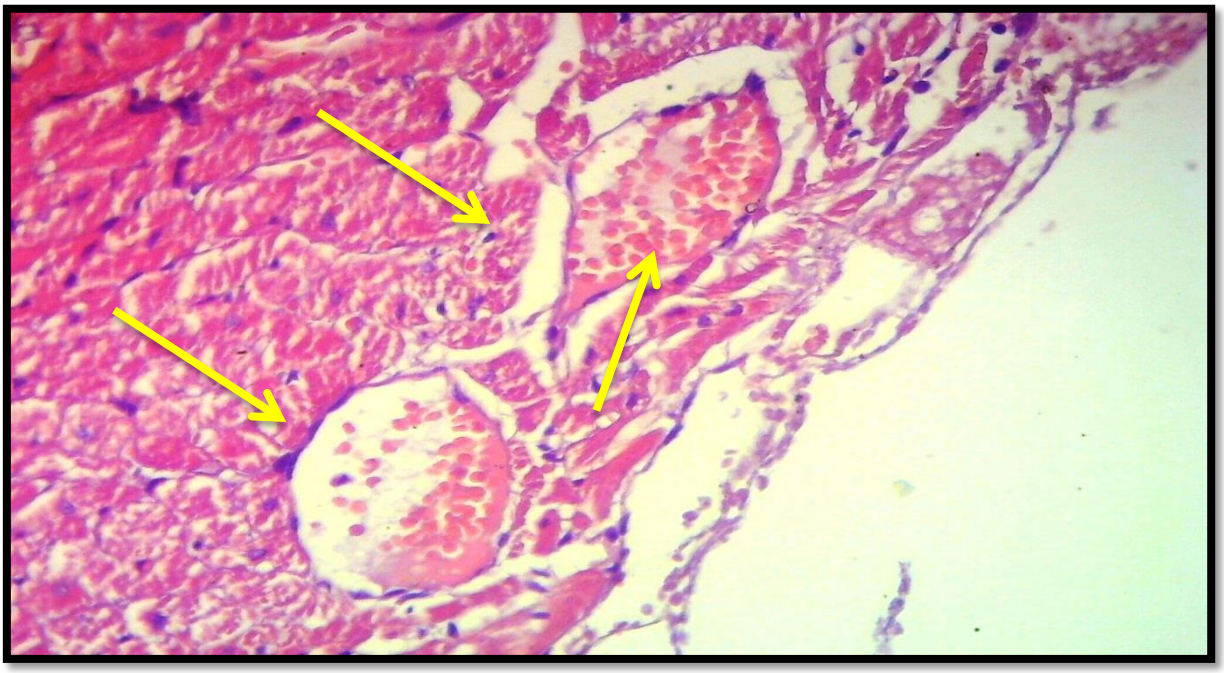

Figure (3): Heart muscle of male rat shows sub capsular congestion of blood vessels. H&E stain 400X.

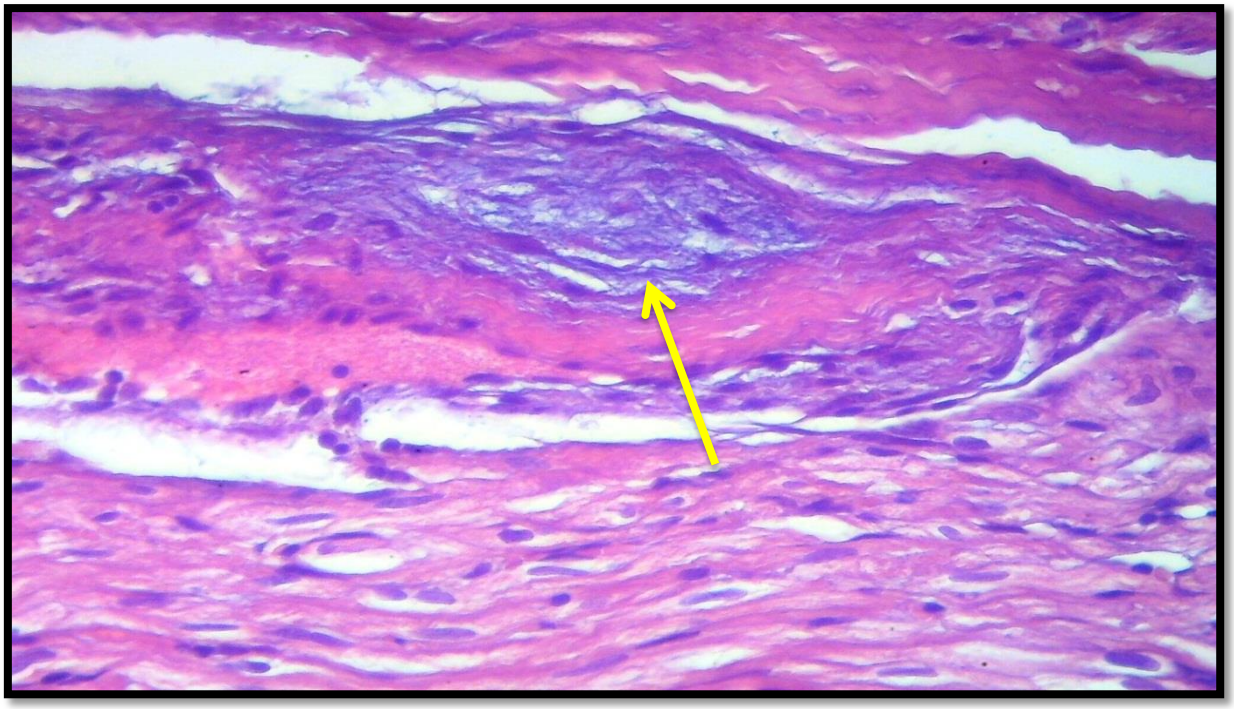

Figure (4): Heart muscle of female rat shows an area of fibrosis in sub capsular region. H&E stain 400X.

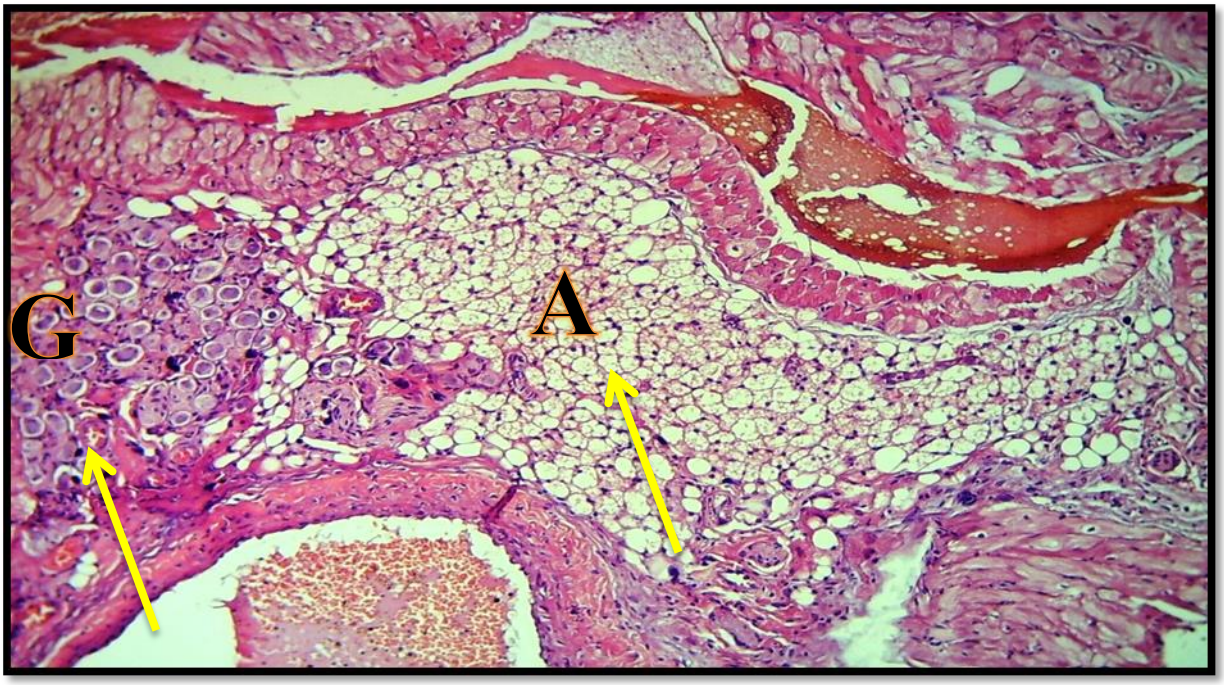

Figure (5): Heart muscle of female rat shows the presence of adipose tissue (A)and groups of ganglia like cells (G) in the pericardial region. H&E stain 100X.

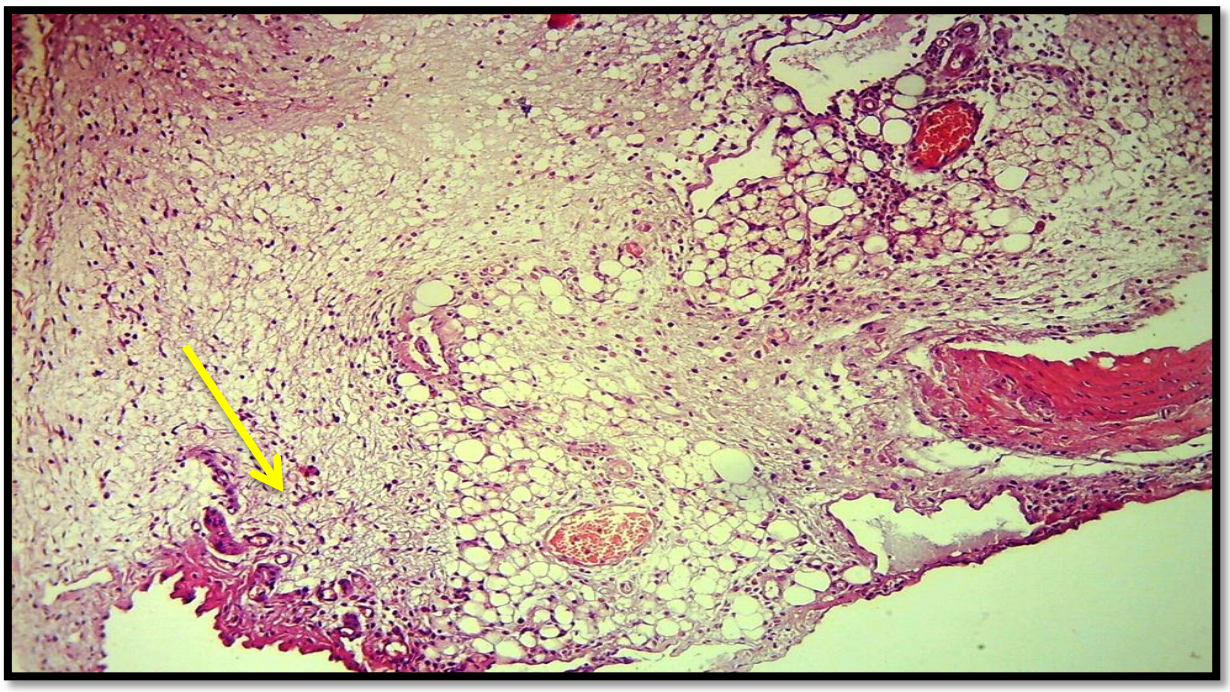

Figure (6): Heart muscle of female rat shows an area sub capsular fibrosis, adipose tissue and some inflammatory cells. H&E stain 100X.

The table showed a significant (P≤0.05) increase in serum troponin level of High group(G4) when compared with Intermediate(G3), Low(G2) and Control(G1) groups as well as show a significant (P≤0.05) increase in serum troponin level in both Intermediate and Low groups in compared with Control group. On the other hands, there is no significant difference in serum troponin level between Intermediate and Low group in treated animals.

Table Show the effect of digoxin on serum troponin level. N = 12 (Mean ± SD).

Groups	Troponin mean
Control (G1)	0.70 ± 0.10 c
Low (G2)	1.36 ± 0.23 b
Intermediate (G3)	1.41 ± 0.16 b
High (G4)	1.58 ± 0.22 a
LSD	0.166

Values expressed in the small latters mean significant differences (P≤0.05) level.

Discussion

The present study shows histopathological changes of heart in figure (1,2) varying degree of degenerated myocardial muscle cells presented as pale areas of homogenous cytoplasm grayish in color, this results are in agreement with **[8, 9]** they found myocardial lesion closely related to high dose of oleander in cardiac glycoside due to blockage of Na-K/ATPase pump associated with increase intracellular Ca^{+2} that lead to increase contraction of cardiac muscle, their degeneration and necrosis.

On the other hand, the damage that occur in myocardial muscle cells are very severe in high dose group compared with other study groups, this result is in line with **[10]** who report that the damage occurs with high dose and intermediate dose groups as compared with low and control groups. This result is approved in the study by measuring troponin enzyme level.

The present study showed a significant increase in troponin enzyme level in group4 (High dose group) as compared with other study groups, this result is in line with **[11]** who found a strong relationship between serum level of troponin and the histopathological changes that occur with acute and chronic disease of heart and myocardial infarction. This elevation of serum troponin level may be due to the damage to myocardial muscle cells that accompany high dose of digoxin toxicity and this is in line with **[10]**.

The figure (3,4) show sub capsular congestion of vessels and sub capsular fibrosis of myocyte, this result is in line with **[10,12]** they report the congestion, hemorrhage that accompany the damage to myocardial cells and this may be due to the toxic effect of digoxin on cardiac muscle.

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